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2. Patent application number
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

GlaxoSmithKline Biologicals s.a.
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810127001

Belgian

~~*see continuation sheet for further applicant(s)*~~

4. Title of the invention — Vaccine Composition

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Patents ADP number *(if you know it)*

Corporate Intellectual Property

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Corporate Intellectual Property (CN9 25.1)
980 Great West Road
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11. We request the grant of a patent on the basis of this application

Signature Michael Lubienski Date 16-Jun-03

M J Lubienski

12. Name and daytime telephone number of person to contact in the United Kingdom: M J Lubienski 020 80474434

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VACCINE COMPOSITION

The present invention relates to the field of vaccines, and, in particular, to immunogenic compositions comprising *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP). The present invention presents immunogenic compositions and combination vaccines comprising PRP wherein the PRP is protected to some degree from immune interference that can occur when PRP is combined with other antigen formulations – particularly formulations comprising DTPa; a well known 'trivalent' combination vaccine comprising Diphtheria toxoid (DT), tetanus toxoid (TT), and acellular *B. pertussis* components [typically comprising detoxified pertussis toxoid (PT) and filamentous haemagglutinin (FHA) with optional pertactin (PRN) and/or agglutinogens 2 and 3], typically adsorbed (at least in part) on aluminium hydroxide adjuvant, for example the marketed vaccine INFANRIX-DTPa™ (GlaxoSmithKline Biologicals) which contains DT, TT, PT, FHA, and PRN antigens, all adsorbed onto aluminium hydroxide adjuvant. A method to reduce interference of PRP in a combination vaccine (comprising, for instance, DTPa) is also presented.

Vaccines that utilise polysaccharides are known in the art. For example a PRP vaccine for the prevention of *Haemophilus influenzae* B infections is based on the *H. influenzae* B capsular oligosaccharide or polysaccharide (PRP) conjugated with a carrier protein. The polysaccharide is a polymer of ribose, ribitol and phosphate. Examples of carrier protein include diphtheria or tetanus toxoid, or an outer membrane protein of *N. meningitidis*. See for example US 4,365,170, US 4,673,574, EP 208375, EP 477508 and EP 161188.

It is desirable to administer such conjugate vaccines with other antigens or vaccines at the same time and this can involve multiple injections. Problems associated with multiple injections include a more complicated administration procedure and a large total injection volume. This is a particularly acute problem when the vaccine is intended for infants. For both the infant and the practitioner it is desirable to inject all necessary antigens in one shot of normal volume, thus rendering the vaccination procedure less

traumatic and painful for the infant, and more efficient and easier to manage for the practitioner.

It has therefore been proposed to combine such polysaccharide conjugate vaccines with other vaccines such as DTPa to produce more elaborate combination vaccines. In addition, the inclusion of further antigens to such a combination vaccine for the prevention of diseases like hepatitis B or Polio has also been proposed (combination vaccines comprising an antigen against hepatitis B and antigens against diphtheria, tetanus and pertussis (HepB, DTPa) have been described in WO 93/24148). See also WO 98/00167 and WO 99/13906 which also disclose DTP-PRP combination vaccines.

It has been found, however, that simple mixing of the components of a combination vaccine is complicated by the fact that not all antigens can be effectively mixed together. The reduction in the immunogenicity of an antigen when combined with other components (as compared to the particular antigen administered alone) is known as interference. It is known, for example, that the extemporaneous mixing of a DTPa combination vaccine with unadjuvanted PRP conjugates results in a reduction of antibody titres to the PRP polysaccharide (WO 97/00697). In addition, WO 97/00697 showed that if PRP conjugate is adsorbed onto aluminium hydroxide, there is a significant reduction of antibody titres to the polysaccharide component. These results indicated that there was interference between the aluminium hydroxide of the DTPa vaccine and PRP. In order to try and minimise this interference in such an extemporaneously-prepared combination vaccine PRP was pre-adsorbed onto aluminium phosphate.

Without wishing to be bound by theory, it is thought that the above interference problem may be as a result of PRP (with a low isoelectric point of less than 2) forming a strong interaction with aluminium hydroxide (with a high isoelectric point). This interaction may mask PRP epitopes from immune competent cells – particularly if the PRP/AlOH interaction forms a network of particles – a phenomenon called flocculation which may be observed visually or by optical microscope.

WO 96/37222 also describes the interference problem. In this case the antigenicity of PRP conjugate is stabilised by adsorbing it and the other DTPa

components onto an aluminium-based adjuvant with a zero point charge of less than 7.2, for instance aluminium phosphate, or aluminium hydroxide to which anion salts have been added to lower its zero point charge from around 10 or 11 to under 7.2.

A problem with using aluminium phosphate entirely for a combination vaccine is that many antigens in a combination vaccine benefit immunologically from being adsorbed onto aluminium hydroxide – for instance pertactin. Many of these antigens (for instance pertactin) cannot be adequately adsorbed onto aluminium phosphate, and become desorbed from aluminium hydroxide if sufficient anion salts are added to reduce its zero point charge under 7.2. Pertactin is one of the most important components in the pertussis vaccine, and a minimisation of adsorption on adjuvant reduces the T-cell response and the potency of the acellular pertussis vaccine as a whole. At pH 6.1 (the typical pH of DTPa vaccines) a 24 hour adsorption step allows more than 90% of pertactin to be adsorbed onto aluminium hydroxide, but less than 50% to be adsorbed onto aluminium phosphate (reducing further when it is combined with other antigens).

There is therefore a technical problem in combination vaccines comprising PRP and antigens adsorbed onto aluminium hydroxide to reduce interference to PRP, yet maintain a significant degree of adsorption of antigens beneficially associated with aluminium hydroxide.

WO 99/48525 provides a solution to the above problem which involves a complex process of adsorbing and mixing antigens in order for PRP to be added with minimised interference.

There is still need, however, for further solutions to the above problem which are advantageously simpler – i.e. involve a single, simple additional process step, or involve the simple addition of a single PRP-protective excipient to the immunogenic composition. The present invention provides such a solution.

DESCRIPTION OF THE INVENTION

This invention relates to a general method by which either extemporaneously-prepared or liquid PRP/DTPa combination vaccines can be made in order to reduce the PRP interference problem whilst being able to maintain a significant degree of adsorption of antigens beneficially associated with the aluminium-based adjuvant on which it is most immunogenic. In so doing, pertussis antigens in combination vaccines of the present invention may be stably retained in their most potent form. The invention further provides immunogenic compositions, vaccines and combination vaccines comprising PRP which is protected to some degree from immune interference. The inventors have found that the above can surprisingly be achieved by incorporating a polyanionic polymer excipient with the vaccine comprising PRP. Without wishing to be bound by theory, the polyanionic polymer can compete with PRP, protecting it from any aluminium hydroxide present in the vaccine, yet surprisingly does not cause antigens already adsorbed to aluminium hydroxide to become significantly desorbed.

Accordingly in one embodiment the present invention provides an immunogenic composition comprising a capsular polysaccharide or oligosaccharide of *Haemophilus influenzae* B (PRP), and a polyanionic polymer.

Although PRP is described throughout this specification, it is envisaged that the same solution may protect other oligosaccharides or polysaccharides with a low isoelectric point (less than 3, preferably less than 2), and therefore wherever PRP is mentioned herein, such other oligosaccharides or polysaccharides may alternatively be included as part of the invention.

The terms "comprising", "comprise" and "comprises" herein is intended by the inventors to be substitutable with the terms "consisting of", "consist of" and "consists of", respectively, in every instance.

In a preferred embodiment, PRP is conjugated to a carrier protein which is a source of T-helper cell epitopes.

Preferred carrier proteins for the polysaccharide or oligosaccharide conjugates of the present invention are tetanus toxoid, diphtheria toxoid, CRM197, an outer membrane protein from a bacteria such as *N. meningitidis*, and protein D from non-typeable *H.*

influenzae (EP594610). Most preferably, PRP is conjugated to tetanus toxoid. The synthesis of *Haemophilus influenzae* type B capsular polysaccharide (PRP) tetanus toxoid (TT) conjugate is described, for example, in WO 97/00697.

The polysaccharide or oligosaccharide conjugates of the invention may be prepared by any known coupling technique. For example the polysaccharide can be coupled via a thioether linkage. This conjugation method relies on activation of the polysaccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated polysaccharide may thus be coupled directly or via a spacer group to an amino group on the carrier protein. Preferably, the cyanate ester is coupled with hexane diamine and the amino-derivatised polysaccharide is conjugated to the carrier protein using heteroligation chemistry involving the formation of the thioether linkage. Such conjugates are described in PCT published application WO93/15760 (Uniformed Services University).

The conjugates can also be prepared by direct reductive amination methods as described in US 4365170 (Jennings) and US 4673574 (Anderson). Other methods are described in EP 161188, EP 208375 and EP 477508.

A further method involves the coupling of a cyanogen bromide activated polysaccharide derivatised with adipic acid hydrazide (ADH) to the protein carrier by carbodiimide condensation. Such conjugation is described in Chu C. *et al.* Infec. Immunity, 1983 **245** 256.

A polyanionic polymer of the present invention is a polymer which, when dissolved in an aqueous medium at pH7, is negatively-charged due to the presence of anionic constitutional repeating units (for example, units containing sulphate, sulphonate, carboxylate, phosphate and borate groups). A constitutional repeating unit or monomer refers to the minimal structural unit of a polymer. The polyanionic polymer may be a polyanionic heteropolymer, comprising two or more different anionic constitutional repeating units, or may be a polyanionic homopolymer, consisting of a single anionic constitutional repeating unit.

The polyanionic polymer of the invention may be a chemical polymer and may comprise anionic constitutional repeating units obtained from a group consisting of:

acrylic acid, methacrylic acid, maleic acid, fumaric acid, ethylsulphonic acid, vinylsulphuric acid, vinylsulphonic acid, styrenesulphonic acid, vinylphenylsulphuric acid, 2-methacryloyloxyethane sulphonic acid, 3- methacryloyloxy-2-hydroxypropanesulphonic acid, 3-methacryl amido-3- methylbutanoic acid, acrylamidomethylpropanesulfonic acid, vinylphosphoric acid, 4-vinylbenzoic acid, 3-vinyl oxypropane-1-sulphonic acid, N-vinylsuccinimidic acid, and salts of the foregoing.

Alternatively, the polyanionic polymer of the invention may (or may not, as this is not a preferred embodiment) be an oligo- or poly-saccharide such as dextran.

Most preferably, the polyanionic polymer of the invention is an oligo- or poly-peptide. Such peptides may be D- or L-peptides (preferably the latter so that peptide is advantageously bio-degradable), and may comprise anionic constitutional repeating units (or monomers) such as L-aspartic acid, D-aspartic acid, L-glutamic acid, D-glutamic acid, non-natural anionic amino acids (or salts or anionic chemical derivatives thereof).

For the purposes of this invention, the polyanionic polymer of the present invention is an oligo- or poly-peptide which has a monomer content of no less than 30, 40, 50, 60, 70, 80, 90 or, preferably, 100% L-aspartic acid and/or L-glutamic acid.

Preferably the polyanionic polymer of the invention consists of, on average, 5-200 monomers, preferably 8-117 monomers, more preferably 15-18 monomers, most preferably 17 monomers (or residues in the case of peptides). As polymers are complex populations of molecules of potentially different lengths, "on average" means that number of monomers or residues is calculated according to the weight-average molecular weight (or M_w) of the polyanionic polymer measured by MALLS divided by the molecular weight of the monomer. Preferably the polydispersity (a measure of homogeneity) of the polyanionic polymer is less than 3.

In a particulaly preferred embodiment the polyanionic polymer of the invention is a poly-L-glutamic acid (PLG) homopolymer. Low molecular weight PLG (less than 6000 M_w, preferably 640-5000) is particularly preferred (for instance PLG with on average 17 residues with a M_w of 2178) for optimal clearance from the body post-administration and to ensure it does not induce an immune response itself in the host.

PLG is a fully bio-degradable polyamino acid (available from Sigma-Aldrich) with a pendent free γ-carboxyl group in each repeat unit (pKa 4.1) and is negatively charged at a pH7, which renders this homopolymer water-soluble and gives it a polyanionic structure.

α-PLG is currently used for two main biomedical applications: drug delivery for cancer therapy (Li et al., Clin. Cancer. Res. 6:2829-2834, 2000) and biological glue (Iwata et al., Biomaterials 19:1869-1876, 1998). It has not been used as an excipient for intramuscular vaccination.

Particularly preferred immunogenic compositions of the invention comprise PRP (preferably conjugated) and polyanionic polymer that are advantageously formulated such that the result of multiplying the concentration of the polyanionic polymer in the composition (in μM) by the net negative charge of the polyanionic polymer at pH 7.0 divided by the amount of PRP present in a 0.5 mL dose of the immunogenic composition (in μg) is 300-6000, preferably 400-4000, more preferably 500-2000, 560-1100, 610-900, 640-800, or 660-700, and most preferably around or exactly 680.

The concentration of the polyanionic polymer in the composition should again be measured according to the M_w of polyanionic polymer used, and is typically in the range 30-2000 μM, preferably 80-1000, 100-500, 150-300, and most preferably around or exactly 200 μM.

The net negative charge at pH7.0 of the polyanionic polymer may be calculated by any suitable means. Again this may be an average property of the polymer, and should be calculated with respect to the M_w of polyanionic polymer used. For instance, a PLG polymer with on average 17 residues should have a net negative charge of 17. Preferably the net negative charge should be at least 8, or at least 17, preferably 8-106, 10-80, 12-60, 14-40, 16-20, and most preferably around or exactly 17.

It is preferred that the polyanionic polymer of the invention has at least on average 1 net negative charge at pH 7.0 per 3 monomers, preferably at least 2 per 3 monomers, and most preferably at least on average 1 net negative charge for each

monomer. The charges may be unevenly arranged over the polymer length, but are preferably evenly spread over the polymer length.

The immunogenic compositions of the invention typically comprise 1-20, preferably 2.5-10, and most preferably around or exactly 5 μg of PRP (preferably conjugated to carrier protein, the weight of which is not counted in the above calculations) per 0.5 mL dose. PRP is most preferably not intentionally adsorbed onto any adjuvant.

In a highly preferred embodiment an immunogenic composition is provided comprising 5 μg of PRP conjugated to a carrier protein (preferably tetanus toxoid) and 218 μg of poly-α-L-glutamic acid sodium (approximately 200 μM) per 0.5 mL human dose, wherein the PLG contains on average 17 glutamic acid residues (preferably with a M_w 2,178, and optionally with a polydispersity of 2.6).

In all the above immunogenic compositions, further excipients may be added to those already mentioned. In particular, PRP may be adsorbed onto aluminium phosphate adjuvant (as described in WO 97/00697 and WO 99/48525) but is preferably unadsorbed. The immunogenic composition may be buffered with any suitable buffer that has a pKa that may stabilise the pH of the composition – typically pH6-7, most preferably pH 6.1. For example Histidine buffer may be used, or, preferably, maleate buffer. In general, buffers (and quantities used) should be selected that do not significantly effect the polyanionic polymer's beneficial effect in the composition. In general if a buffer is present, less than 5, 4, 3, 2, 1, 0.5 or 0.1 mM buffer should be used, preferably around 2mM.

If the immunogenic composition of the invention is to be lyophilised for storage purposes, it is preferable that a stabilizing excipient (or cryoprotector) is added to the composition. Any such excipient may be used such as glucose, maltulose, iso-maltulose, lactulose, sucrose, sorbitol, maltose, lactose, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, and melezitose, but preferably sucrose is used. Such excipients are typically present in the amount of 1-5%, and preferably around or exactly 2.5% (w/v).

Although the immunogenic compositions of the invention may have an antigenic content consisting of only PRP (preferably conjugated to a protein carrier), it may comprise one or more further antigens. PRP may be mixed and stored with these other antigens, or may have them added extemporaneously (by a practitioner just before administering the composition to a patient in need thereof). The polyanionic polymer may be added to the one or more other antigens before PRP is combined with them, or, preferably is present with the PRP as a protectant before the other antigens are combined with it. Some of the further antigens may be stored with PRP (preferably lyophilised) and some stored separately (preferably liquid) to be reconstituted together extemporaneously, wherein the polyanionic polymer may be present in either composition, but is preferably present with the PRP.

Preferably, the immunogenic composition in addition to PRP (preferably conjugated) and polyanionic polymer further comprises one or more meningococcal capsular oligosaccharide or, preferably, polysaccharide – carrier protein conjugates (see above for preferred carrier proteins comprising T-helper epitopes, most preferably tetanus toxoid) selected from a group consisting of: MenC, MenY, MenA and MenW; preferably MenC and/or MenY is included, and most preferably all 4. These meningococcal components are preferably not intentionally adsorbed onto any adjuvant. Such immunogenic compositions are beneficially lyophilised, and may be reconstituted with further antigens (for instance DTPa-based compositions), preferably extemporaneously.

Alternatively or in addition to the above meningococcal antigens, the immunogenic composition may comprise one or more pneumococcal capsular oligosaccharide or polysaccharide – carrier protein conjugates (see above for preferred carrier proteins comprising T-helper epitopes, most preferably CRM197 or diphtheria toxoid).

Typically pneumococcal capsular oligosaccharides or polysaccharides (preferably the latter) represented in the compositions of the invention comprise antigens derived from at least four serotypes of pneumococcus. Preferably the four serotypes comprise 6B, 14, 19F and 23F. More preferably, at least 7 serotypes are comprised in the

composition, for example those derived from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. More preferably still, at least 11 serotypes are comprised in the composition (11 valent), for example those derived from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. In a preferred embodiment of the invention at least 13 of such conjugated pneumococcal antigens are comprised, although further antigens, for example 23 valent (such as serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F), are also contemplated by the invention.

For elderly vaccination (for instance for the prevention of pneumonia) it is advantageous to include serotypes 8 and 12F (and most preferably 15 and 22 as well) to the 11 valent pneumococcal antigenic composition described above to form a 15 valent composition, whereas for infants or toddlers (where otitis media is of more concern) serotypes 6A and 19A are advantageously comprised to form a 13 valent composition.

Again such immunogenic compositions comprising pneumococcal antigens are beneficially lyophilised, and may be reconstituted with further antigens (for instance DTPa-based compositions), preferably extemporaneously.

The immunogenic composition of the invention in addition to PRP and polyanionic polymer may further comprise one or more (2 or preferably all 3 [a DTPw or DTPa composition]) further antigens or antigen groups selected from tetanus toxoid (TT), diphtheria toxoid (DT), and whole-cell or one or more acellular *B. pertussis* antigens. The one or more (2, 3, 4 or all 5) acellular *B. pertussis* antigens that may be comprised may be selected from the group consisting of: pertussis toxoid (PT), FHA, pertactin (PRN), agglutinogen 2 and agglutinogen 3 (and preferably comprises the first three).

Such DTPa or DTPw compositions may further comprise either or both of Inactivated Polio Vaccine (IPV) (typically unadsorbed) and Hepatitis B surface antigen (which is preferably adsorbed onto aluminium phosphate as described in WO 93/24148).

DT, TT, PT, FHA and PRN are well known in the art. The PT component may be made into a toxoid either chemically or genetically, for example as described in EP 515415. See also EP 427462 and WO 91/12020 for the preparation of pertussis antigens.

Optionally the PT component may be recombinant (for example as described in European Patent Applications EP 306318, EP 322533, EP 396964, EP 322115 and EP 275689). Optionally the DT and TT components may also be recombinant. Typically the PT, FHA, PRN, HBsAg (Hepatitis B surface antigen), and PRP components will be in the range 8-25 μg per 0.5 mL dose of bulk vaccine. The DT, TT, and IPV (inactivated trivalent poliovirus vaccine) components should typically be present as approximately 15-25 Lf (flocculating units), 10 Lf, and 40/8/32 (type I/II/III) DU respectively per 0.5 mL dose of bulk vaccine.

Suitable components for use in such vaccines are already commercially available and details may be obtained from the World Health Organisation. For example the IPV component may be the Salk inactivated polio vaccine. The Hepatitis B surface antigen may comprise the 'S' antigen as in Engerix-B™ (SmithKline Beecham Biologicals).

The addition of either lyophilised or liquid PRP (either unadjuvanted or adsorbed onto aluminium phosphate) to a solution of the other components of the composition may be performed extemporaneously, or before the vaccine leaves the manufacturer. PRP may be combined with polyanionic polymer before its addition to the other components, or PRP may be added to other components further comprising polyanionic polymer.

The immunogenic compositions of the invention will typically further comprise an adjuvant with a zero point charge greater than 8, 9 or 10, typically an aluminium salt, most often alum or aluminium hydroxide. This will particularly be the case for DTPa-containing compositions where one or more DTPa component is preferentially adsorbed onto aluminium hydroxide. Usually such adjuvant is present in the immunogenic composition in the amount of 100-1000 μg per 0.5 mL dose, usually around or exactly 500 μg per 0.5 mL dose, of which around 50, 60, 70, 80, 90 or 95% has antigen (non-PRP, usually one or more DTPa antigens) specifically adsorbed onto its surface.

The presence of such adjuvants would normally tend to flocculate with any PRP present in the composition, however in the immunogenic compositions of the present invention this is prevented by the presence of the polyanionic polymer.

Alternatively, and preferably additionally, the presence of the polyanionic polymer can reduce the immunological interference that the adjuvant has on PRP by over 20, 30, 40, 50, 60, 70, 80, 90, or, preferably, by 100% (interference being measured by taking the difference in anti-PRP GMT titres in (μg/mL) in an appropriate model [e.g. mouse or rat, or in a well-conducted human clinical trial] between administering a PRP vaccine by itself versus the same PRP vaccine in an immunogenic composition comprising the above adjuvant; the reduction of interference being the extent to which the GMT in the immunogenic composition is restored to that of the PRP vaccine by itself by the addition of the polyanionic polymer of the invention to the immunogenic composition).

Where the above adjuvant (with a zero-point charge greater than 8) is included in the immunogenic compositions of the invention, this is usually because certain antigens in the composition are most effective when adsorbed to the surface of such adjuvants (particularly aluminium hydroxide).

An advantage of the immunogenic compositions of the invention is that the presence of the polyanionic polymer (unlike regular anionic salts) does not cause significant desorption of antigens that are specifically adsorbed onto the above adjuvant (i.e. with a zero-point charge greater than 8). By not causing "significant desorption" it is typically meant that more than 50, 60, 70, 80 or, preferably, 90 % of antigen that has been specifically adsorbed onto the adjuvant remain adsorbed to the adjuvant after 1 hour of adding the polyanionic polymer to the immunogenic composition of the invention. In general it is preferred that in so doing sufficient antigen remains adsorbed onto the adjuvant in order for the anti-antigen GMT titre in (μg/mL) in an appropriate model [e.g. mouse or rat, or in a well-conducted human clinical trial] to be more than 50, 60, 70, 80, 90, or 95 % of the GMT titre of the antigen in the same immunogenic composition without polyanionic polymer in the same model.

Typically one or more of the following antigens may be present in the immunogenic composition of the invention and may have been specifically (and preferably individually) adsorbed onto an adjuvant with a zero point charge more than 8 (preferably aluminium hydroxide) before mixing with the other components of the

immunogenic composition of the invention: diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA and pertactin. Preferably at least PRN is adsorbed onto aluminium hydroxide, and most preferably all 5 of the components are adsorbed onto aluminium hydroxide.

Methods of adsorbing DTPa antigens onto aluminium adjuvants are known in the art. See for example WO 93/24148 and WO 97/00697. Usually components adsorbed onto adjuvant are left for a period of at least 10 minutes at room temperature at an appropriate pH for adsorbing most and preferably all of the antigen before mixing the antigens together in the combination immunogenic compositions of the present invention.

Other components are preferably unadsorbed (such as IPV) or adsorbed specifically onto other adjuvants - Hepatitis B surface antigen being preferably adsorbed onto aluminium phosphate (as described in WO 93/24148) before mixing with other components.

In a preferred embodiment, a vaccine is provided comprising the immunogenic composition of the invention and a pharmaceutically acceptable excipient. The pH of the vaccines of the present invention is usually between pH6-7, preferably pH 6.1.

Vaccine preparation is generally described in Vaccine Design - The Subunit and adjuvant approach Ed Powell and Newman; Pellum Press. Advantageously the combination vaccine according to the invention is a paediatric vaccine.

The amount of polysaccharide or oligosaccharide conjugate antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending on which specific immunogens are employed. Generally it is expected that each dose will comprise 1-1000 μg of conjugated polysaccharide or oligosaccharide (expressed in amount of saccharide), preferably 2-100 μg, and most preferably 4-40 μg.

The content of protein antigens in the vaccine will typically be in the range 1-100μg, preferably 5-50μg, most typically in the range 5 - 25μg.

An optimal amount of antigen for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive one or two booster injections at about 4 weeks intervals or longer.

The vaccine preparations of the present invention may be used to protect or treat a mammal (preferably human) susceptible to infection, by means of administering said vaccine via systemic or mucosal route. These administrations may include injection *via* the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or (less preferred) *via* mucosal administration to the oral/alimentary, respiratory, genitourinary tracts..

There is further provided a method of preventing or treating *H. influenzae* B disease by administering a pharmaceutically effective amount of the vaccine of the invention to a patient in need thereof, and a use of the immunogenic composition or vaccine of the invention in the manufacture of a medicament for the prevention or treatment of *H. influenzae* B disease.

The present invention additionally provides a method for reducing the immunological interference of a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), which is preferably conjugated, in a combination vaccine (a vaccine of the invention comprising PRP and at least one further antigen) comprising one or more further antigens adsorbed to an adjuvant with a zero point charge greater than 8 (as described above, preferably aluminium hydroxide), wherein such method comprises the steps of:

(i) adsorbing the one or more further antigens onto the adjuvant;

(ii) adding a polyanionic polymer to said one or more further antigens; and

(iii) then adding an immunogenic composition comprising PRP to said one or more further antigens;

or comprising the steps of:

(i) adsorbing the one or more further antigens onto the adjuvant; and

(ii) adding an immunogenic composition of the invention comprising PRP and a polyanionic polymer to said one or more further antigens.

In the former the adjuvant is policed before PRP is added, in the latter PRP is protected before it is added to the adjuvant. Preferably, in either method the components are mixed extemporaneously. The immungenic composition comprising PRP is preferably lyophilised for greatest stability, most preferably in the presence of a stabilizing excipient (as described above). The immungenic composition comprising PRP is preferably combined with one or more conjugated meningococcal capsular oligosaccharides or polysaccharides and/or one or more conjugated pneumococcal capsular oligosaccharides or polysaccharides (as described above).

Preferably the one or more further antigens adsorbed to adjuvant are selected from diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA and pertactin, most preferably all, as described above. Most preferably, as described above, the presence of the polyanionic polymer in the combination vaccine does not cause significant desorption of the one or more further antigens adsorbed to the adjuvant.

Further provided is a use of a polyanionic polymer (as described above) in an immunogenic composition further comprising a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), preferably conjugated, as a means for protecting the immune response of PRP. By protecting the immune response it is meant retaining more than 50, 60, 70, 80, 90, or 95% of the anti-PRP GMT titre of the PRP component by itself, regardless of whether the immunogenic composition is later combined with a vaccine comprising adjuvant with a zero-point charge greater than 8 (as described above).

A kit is further provided comprising: i) a first immunogenic composition comprising a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), preferably conjugated, and a polyanionic polymer (as described above); and ii) a second immunogenic composition comprising one or more antigens adsorbed onto an adjuvant with a zero point charge greater than 8 (preferably aluminium hydroxide). Preferably, the first immunogenic composition is lyophilised and further comprises a

stabilizing excipient (as described above), preferably sucrose, and the second immunogenic composition is liquid. It is envisaged the contents of the kit may be simply administered by extemporaneously reconstituting the first immunogenic composition with the second immunogenic composition, and administering the resulting mixed composition. It is highly preferred that the polyanionic polymers of the present invention can dissolve in aqueous solution faster than PRP or PRP conjugates so that, when co-lyophilised, the polymer (such as PLG) may effectively protect the slower dissolving PRP when it is reconstituted in a liquid composition comprising an adjuvant with a zero point charge greater than 8.

Preferably the first immunogenic composition further comprises one or more conjugated meningococcal capsular oligosaccharides or polysaccharides selected from a group consisting of: MenC, MenY, MenA and MenW, preferably MenC and/or MenY, and/or one or more conjugated pneumococcal capsular oligosaccharides or polysaccharides (as described above). Preferably, the second immunogenic composition comprises one or more (most preferably all) antigens selected from a group consisting of: diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA and pertactin.

The following examples illustrate, but do not the limit the scope of, the invention.

Examples

Numerous experiments were done with PRP polysaccharide conjugated to tetanus toxoid (PRP-T) in conjunction with PLG (Hib-PLG) experimental lots. The following parameters were evaluated:

- the molecular weight and content of PLG,
- the content of PRP-T,
- the stabiliser for the lyophilisation.

The following results are from the *in-vitro*, pre-clinical and potency testing of these experimental lots.

Results with the experimental lots

In-vitro data with Hib-PLG

Three types of mixing steps were followed to demonstrate the efficacy of PLG in reducing the physical interaction between PRP-T and $Al(OH)_3$, including mixing the commercially available Infanrix Penta vaccine with Hib-PLG vaccine:

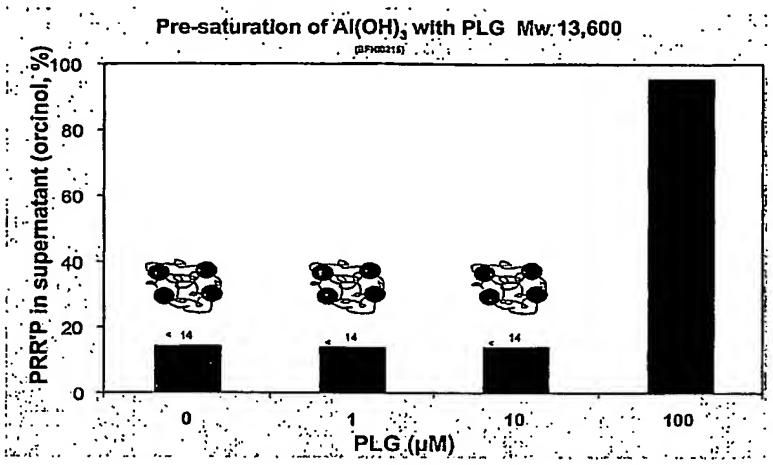
- Step 1: pre-saturation whereby PLG was first adsorbed on $Al(OH)_3$, then PRP-T was added.
- Step 2: competition whereby PLG was put in competition with PRP-T for adsorption on $Al(OH)_3$
- Infanrix Penta/Hib-PLG: whereby PLG and PRP-T were co-lyophilized and then put in competition for adsorption in PeNTa vaccine, containing 500µg $Al(OH)_3$

In all 3 steps, PLG was able to avoid flocculation induced by PRP-T as well as its adsorption on $Al(OH)_3$ (see Figures 1 and 2).

The 200µM PLG (Mw 2,200) content was selected for clinical formulation as:

- no flocculation was observed,
- close to 80% PRP-T was non-adsorbed (according to the Dionex test)
- PLG is fully adsorbed (preliminary results)
- all major components of Infanrix (DT, TT, PT, FHA, PRN [or 69K], IPV and HB surface antigen) were not affected.
- Both lactose and sucrose were found to be efficient cryoprotectors.

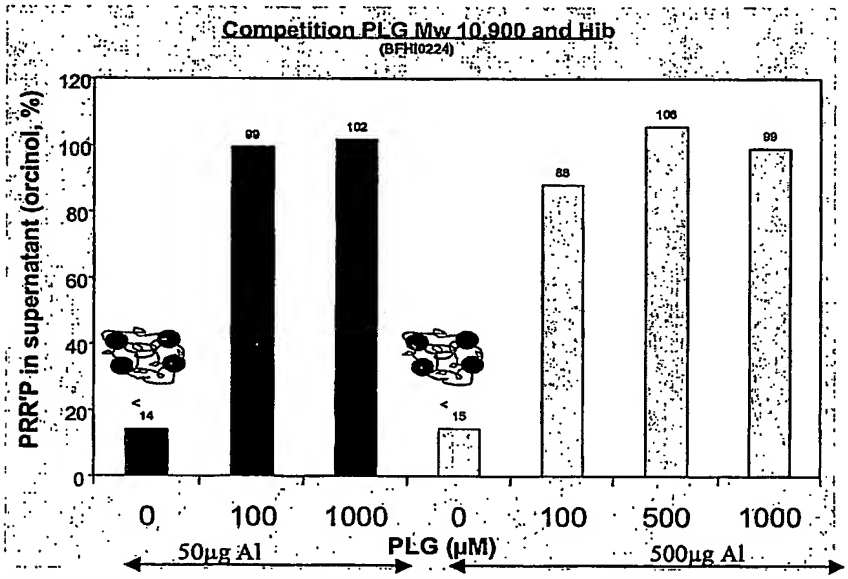
Figure 1 : Pre-saturation of $Al(OH)_3$ with PLG (106 residues)



10 ug PRP/dose, 500 ug AlOH

It was also found that 2000 uM PLG (Mw 1043 – 8 residues) could keep 80% of PRP-T in the supernatant (10 ug PRP/dose, 500 ug AlOH) with no flocculation resulting

Figure 2 : Competition between PRP-T and PLG (85 residues)



100µM PLG Mw 10,900 are able to limit adsorption of 10µg Hib on 50 (to mimic hypothetical free $Al(OH)_3$ in Infanrix PeNTa) as well as on 500µg $Al(OH)_3$ (= full $Al(OH)_3$ dose in Infanrix PeNTa)

In addition, 500µm PLG Mw 2,178 (17 res.) are able to limit adsorption of 10µg Hib on $Al(OH)_3$ after reconstitution of [Hib-PLG] cake with Infanrix PeNTa (1h

contact, then centrifugation 6min 6500g and dosage of Hib in supernatant by ELISA PRR'P-TT or Dionex dosage) , as can 75µM PLG Mw 10,900 (85 res.).

PLG Mw 2,178 (17 res.) is able to limit adsorption of 5µg Hib (PRP-T) on $Al(OH)_3$ after reconstitution of [Hib-PLG] cake with Infanrix PeNTa (1h contact, then centrifugation 6min 6500g and dosage of Hib in supernatant by ELISA PRR'P-TT or Dionex dosage) - 175 and 200µM were optimal concentrations in that there is an absence of flocculation and Infanrix antigen adsorption is retained.

PLG Mw 10,800 (85 residues) is able to limit adsorption of 5µg Hib (amount of PRP in PRP-T) on $Al(OH)_3$ after reconstitution of [Hib-PLG] cake with Infanrix PeNTa (1h contact, then centrifugation 6min 6500g and dosage of Hib in supernatant by ELISA PRR'P-TT or Dionex dosage) - 30 and 35µM were optimal concentrations in that there is an absence of flocculation and Infanrix antigen adsorption is retained.

Pre-clinical immunogenicity data with Hib-PLG

Hib-PLG experimental formulations were evaluated in a rabbit model of immunogenicity and a baby rat model allowing evaluation of the Hib (PRP-T conjugate) immune interference induced by combination of Infanrix Penta and Hib vaccines. Moreover, impact of Hib-PLG on the efficacy of Infanrix-Penta was evaluated in a *B. pertussis* lung colonization murine model.

Rabbit model of immunogenicity

Study design

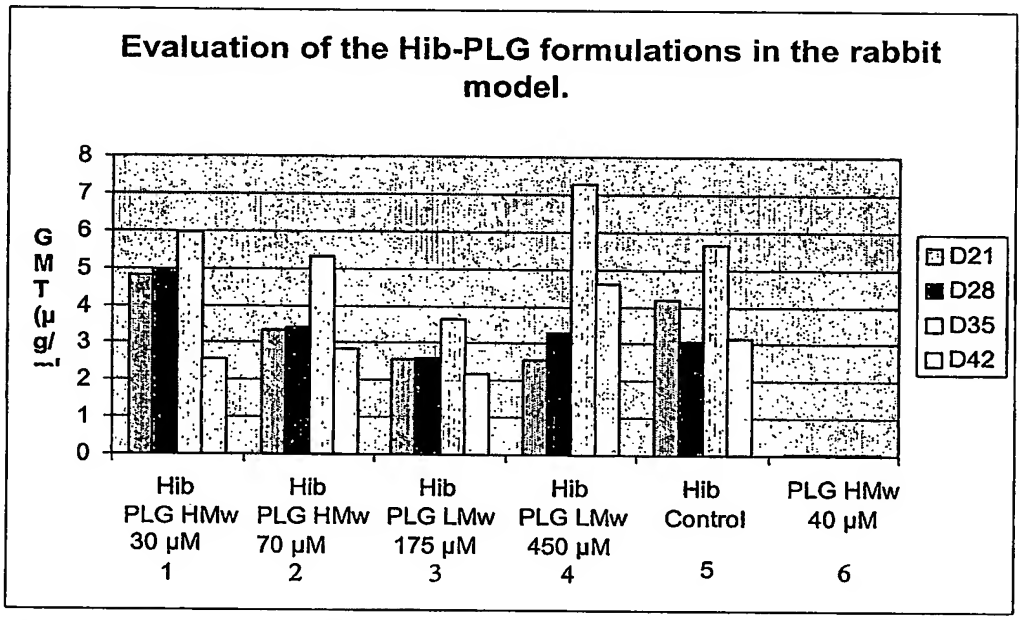
In this model, 5 week old New Zealand female rabbits were intramuscularly immunised three times at two weeks intervals (day 0, 14, 28) with ¼ of a vaccine human dose. A sample size of 10 animals per group was used. Blood samples were taken on day 21, 28, 35 and 42. Anti-PRP antibodies were measure by ELISA and are expressed in µg/ml.

Vaccines administered

Formulations of PRP-T (5µg PRP) containing high (10900 MW) and low (2200 MW) molecular weight PLG were evaluated in two concentrations. A similar Hib formulation but without PLG was included as control. See below for details of vaccines administered by group.

Group	Vaccine
1	Hib (5µg) PLG HMW (10900) 30µM
2	Hib (5µg) PLG HMW (10900) 70µM
3	Hib (5µg) PLG LMW (2200) 175µM
4	Hib (5µg) PLG LMW (2200) 450µM
5	Hib (5µg)
6	PLG HMW (10900) 40µ

Results



Although some variability of PRP response can be observed in this rabbit model, no significant difference was demonstrated between groups.

There was a slight reduction of immunogenicity observed in rabbits having received Hib-PLG L Mw 175 µM, but all other formulations induced anti-PRP antibody levels similar to the Hib control group without addition of PLG.

No induction of anti-PLG antibodies after immunization of rabbits with these Hib-PLG experimental formulations were demonstrated.

Baby rat model of Hib interference

Study design

Seven day old OFA rats were intramuscularly immunised three times at two weeks intervals (day 0, 14, 28) with 1/10 of a vaccine human 0.5 mL dose. An equal repartition of male and female rats was realised. A sample size of 20 animals per group was used. Blood samples were realised on day 35 and anti-PRP antibodies were measured by ELISA and expressed in µg/ml.

Vaccines administered

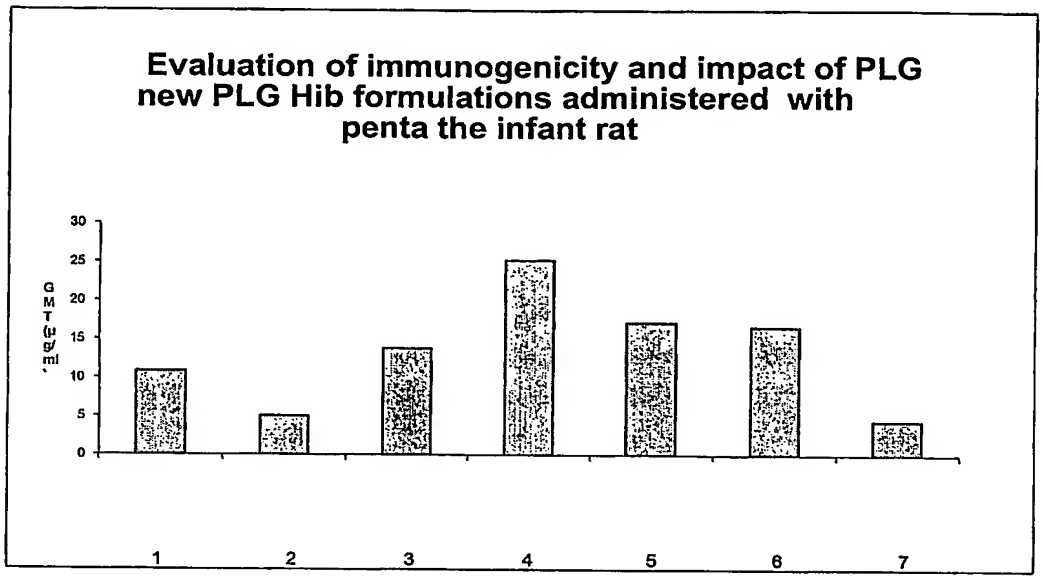
As a control of interference in the baby rat model, Hib combined with Infanrix-Penta (10 µg PRP) was administered as well as Hib (10µg) co-administered with Infanrix Penta. Hib (5 µg) formulated alone or containing various amounts of PLG were evaluated after reconstitution with Infanrix Penta.
See below for details of vaccines administered by group

Group	Vaccine
1	Hib (10µg) + Infanrix Penta
2	Hib (10µg) reconstituted with Infanrix Penta
3	Hib (5µg) PLG HMW (10900) 30µM reconstituted with Infanrix Penta
4	Hib (5µg) PLG HMW (10900) 75µM reconstituted with Infanrix Penta
5	Hib (5µg) PLG LMW (2200) 175µM reconstituted with Infanrix Penta
6	Hib (5µg) PLG LMW (2200) 500µM reconstituted with Infanrix Penta
7	Hib (5µg) reconstituted with Infanrix Penta

Results

When Hib is administered with Infanrix-Penta immune interference was observed as compared with Hib co-administered separately with Infanrix-Penta.

The presence of PLG in Hib formulations resulted in partial or total restoration of the anti-Hib response. Indeed, a higher immune response against PRP was observed in all Hib- formulations containing PLG compared to the control group (Group 7).



These results demonstrate that prevention of Hib interaction with adsorption on $Al(OH)_3$ by addition of PLG can restore high anti-PRP antibody titres elicited by monovalent Hib vaccine.

No induction of anti-PLG antibodies after immunization of baby rats with these Hib-PLG experimental formulations were demonstrated.

Claims

We claim:

1. An immunogenic composition comprising a capsular polysaccharide or oligosaccharide of *Haemophilus influenzae* B (PRP), and a polyanionic polymer.

2. The immunogenic composition of claim 1, wherein PRP is conjugated to a carrier protein which is a source of T-helper cell epitopes.

3. The immunogenic composition of claim 2, wherein the carrier protein is selected from the group consisting of: tetanus toxoid, diphtheria toxoid, CRM197, and protein D.

4. The immunogenic composition of claims 1-3, the polyanionic polymer having anionic constitutional repeating units.

5. The immunogenic composition of claims 1-4, wherein the polyanionic polymer comprises anionic constitutional repeating units obtained from a group consisting of: acrylic acid, methacrylic acid, maleic acid, fumaric acid, ethylsulphonic acid, vinylsulphuric acid, vinylsulphonic acid, styrenesulphonic acid, vinylphenylsulphuric acid, 2-methacryloyloxyethane sulphonic acid, 3- methacryloyloxy-2-hydroxypropanesulphonic acid, 3-methacryl amido-3- methylbutanoic acid, acrylamidomethylpropanesulfonic acid, vinylphosphoric acid, 4-vinylbenzoic acid, 3-vinyl oxypropane-1-sulphonic acid, N-vinylsuccinimidic acid, and salts of the foregoing.

6. The immunogenic composition of claims 1-4, wherein the polyanionic polymer is an oligo- or poly-saccharide such as dextran.

7. The immunogenic composition of claims 1-4, wherein the polyanionic polymer is an oligo- or poly-peptide and comprises anionic constitutional repeating units obtained

from a group consisting of: L-aspartic acid, D-aspartic acid, L-glutamic acid, D-glutamic acid, and salts of the foregoing.

8. The immunogenic composition of claim 7, wherein the polyanionic polymer is an oligo- or poly-peptide which has a monomer content of no less than 30, 40, 50, 60, 70, 80, 90 or 100% L-aspartic acid and/or L-glutamic acid.

9. The immunogenic composition of claim 7 or 8, wherein the oligo- or polypeptide consists of, on average, 5-200 residues, preferably 8-117 residues, more preferably 15-18 residues, most preferably 17 residues.

10. The immunogenic composition of claims 1-9, wherein the polyanionic polymer is polyanionic heteropolymer.

11. The immunogenic composition of claim 10, wherein the polyanionic heteropolymer consists of two distinct anionic constitutional repeating units.

12. The immunogenic composition of claims 1-9, wherein the polyanionic polymer is a polyanionic homopolymer.

13. The immunogenic composition of claim 12, wherein the polyanionic polymer is poly-L-glutamic acid (PLG).

14. The immunogenic composition of claims 1-13, wherein the result of multiplying the concentration of the polyanionic polymer (in μM) by the net negative charge of the polyanionic polymer at pH 7.0 divided by the amount of PRP present in a 0.5 mL dose of the immunogenic composition (in μg) is 300-6000, preferably 400-4000, more preferably 500-2000, 560-1100, 610-900, 640-800, or 660-700, and most preferably around or exactly 680.

15. The immunogenic composition of claims 1-14, wherein the concentration of the polyanionic polymer in the composition is 30-2000 in μM.

16. The immunogenic composition of claims 1-15, wherein the polyanionic polymer has a net negative charge at pH 7.0, on average, of at least 8, and preferably at least 17.

17. The immunogenic composition of claims 1-16, wherein the polyanionic polymer has at least on average 1 net negative charge at pH 7.0 per 3 monomers, preferably at least 2 per 3 monomers, and most preferably at least on average 1 net negative charge for each monomer.

18. The immunogenic composition of claims 1-17, wherein the amount of PRP present in a 0.5 mL dose of the immunogenic composition is 1-20, preferably 2.5-10, and most preferably around or exactly 5 μg.

19. The immunogenic composition of claims 1-18, wherein the immunogenic composition comprises one or more further antigens.

20. The immunogenic composition of claim 19, wherein the one or more further antigens comprise one or more meningococcal capsular oligosaccharide or polysaccharide – carrier protein conjugates selected from a group consisting of: MenC, MenY, MenA and MenW, preferably MenC and/or MenY.

21. The immunogenic composition of claim 19 or 20, wherein the one or more further antigens comprise one or more pneumococcal capsular oligosaccharide or polysaccharide – carrier protein conjugates.

22. The immunogenic composition of claim 20 or 21, wherein the carrier protein is selected from the group consisting of: tetanus toxoid, diphtheria toxoid, CRM197, and protein D.

23. The immunogenic composition of claims 19-22, wherein the one or more further antigens comprise tetanus toxoid, diphtheria toxoid, and whole-cell or one or more acellular *B. pertussis* antigens.

24. The immunogenic composition of claims 19-23, wherein the one or more further antigens comprise one or more acellular *B. pertussis* antigens selected from the group consisting of: pertussis toxiod, FHA, pertactin, agglutinogen 2 and agglutinogen 3.

25. The immunogenic composition of claims 19-24, wherein the one or more further antigens comprise either or both of Inactivated Polio Vaccine (IPV) and Hepatitis B surface antigen, wherein Hepatitis B surface antigen is preferably adsorbed onto aluminium phosphate.

26. The immunogenic composition of claims 19-25, which further comprises an adjuvant with a zero point charge greater than 8; wherein the polyanionic polymer prevents flocculation between the adjuvant and PRP and/or reduces the immunological interference that the adjuvant has on PRP.

27. The immunogenic composition of claim 26, wherein the adjuvant is selected from the group consisting of: alum and aluminium hydroxide.

28. The immunogenic composition of claim 26 or 27, wherein the adjuvant is present in the immunogenic composition in the amount of 100-1000 μg per 0.5 mL dose.

29. The immunogenic composition of claims 26-28, wherein at least one of the one or more further antigens is adsorbed onto the adjuvant.

30. The immunogenic composition of claim 29, wherein the presence of the polyanionic polymer does not cause significant desorption of the one or more further antigens adsorbed onto the adjuvant.

31. The immunogenic composition of claim 29 or 30, comprising the following antigens adsorbed onto aluminium hydroxide: diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA and pertactin.

32. The immunogenic composition of claim 31, further comprising unadsorbed IPV, and Hepatitis B surface antigen adsorbed onto aluminium phosphate.

33. The immunogenic composition of claims 1-32, which is lyophilised and further comprises a stabilizing excipient selected from the group consisting of: glucose, maltulose, iso-maltulose, lactulose, sucrose, sorbitol, maltose, lactose, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, and melezitose; preferably sucrose.

34. A vaccine comprising the immunogenic composition of claims 1-33 and a pharmaceutically acceptable excipient.

35. A method of preventing or treating *H. influenzae* B disease comprising the steps of administering a pharmaceutically effective amount of the vaccine of claim 34 to a patient in need thereof.

36. The use of the immunogenic composition of claims 1-33 or the vaccine of claim 34 in the manufacture of a medicament for the prevention or treatment of *H. influenzae* B disease.

37. A method to reduce the immunological interference of a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), preferably conjugated, in a

combination vaccine comprising one or more further antigens adsorbed to an adjuvant with a zero point charge greater than 8, wherein such method comprises the steps of:

(i) adsorbing the one or more further antigens onto the adjuvant;

(ii) adding a polyanionic polymer to said one or more further antigens; and

(iii) then adding an immunogenic composition comprising PRP to said one or more further antigens.

38. A method to reduce the immunological interference of a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), preferably conjugated, in a combination vaccine comprising one or more further antigens adsorbed to an adjuvant with a zero point charge greater than 8, wherein such method comprises the steps of:

(i) adsorbing the one or more further antigens onto the adjuvant; and

(ii) adding an immunogenic composition comprising PRP and a polyanionic polymer to said one or more further antigens.

39. The method of claim 37 or 38 wherein the immunogenic composition is added extemporaneously to said one or more further antigens.

40. The method of claims 37-39, wherein the immunogenic composition is lyophilised in the presence of a stabilizing excipient, preferably sucrose.

41. The method of claims 37-40, wherein the immunogenic composition further comprises one or more conjugated meningococcal capsular oligosaccharides or polysaccharides selected from a group consisting of: MenC, MenY, MenA and MenW, preferably MenC and/or MenY.

42. The method of claims 37-41, wherein the immunogenic composition further comprises one or more conjugated pneumococcal capsular oligosaccharides or polysaccharides.

43. The method of claims 37-42, wherein the adjuvant is aluminium hydroxide.

44. The method of claims 37-43, wherein the one or more further antigens comprise the following antigens: diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA and pertactin.

45. The method of clims 37-44, wherein the presence of the polyanionic polymer in the combination vaccine does not cause significant desorption of the one or more further antigens adsorbed to the adjuvant.

46. The use of a polyanionic polymer in an immunogenic composition further comprising a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), preferably conjugated, as a means for protecting the immune response of PRP.

47. A kit comprising: i) a first immunogenic composition comprising a *Haemophilus influenzae* B capsular polysaccharide or oligosaccharide (PRP), preferably conjugated, and a polyanionic polymer; and ii) a second immunogenic composition comprising one or more antigens adsorbed onto an adjuvant with a zero point charge greater than 8.

48. The kit of claim 47, wherein the first immunogenic composition is lyophilised and further comprises a stabilizing excipient, preferably sucrose, and the second immunogenic composition is liquid.

49. The kit of claim 47 or 48, wherein the first immunogenic composition further comprises one or more conjugated meningococcal capsular oligosaccharides or polysaccharides selected from a group consisting of: MenC, MenY, MenA and MenW, preferably MenC and/or MenY.

50. The kit of claims 47-49, wherein the first immunogenic composition further comprises one or more conjugated pneumococcal capsular oligosaccharides or polysaccharides.

51. The kit of claims 47-50, wherein the adjuvant is aluminium hydroxide.

52. The kit of claims 47-51, wherein the second immunogenic composition comprises one or more antigens selected from a group consisting of: diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA and pertactin.

ABSTRACT

The present invention relates to the field of vaccines, and in particular vaccines comprising the capsular polysaccharide or oligosaccharide of *H. influenzae* B (PRP). Immunogenic compositions and methods of making such compositions are presented in which the PRP is surprisingly protected from immune interference by adding a polyanionic polymer to the composition.